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Nociceptive evaluation of the association between physical exercises and platelet-rich fibrin in Wistar rats submitted to median nerve compression

Avaliação nociceptiva da associação entre exercício físico e fibrina rica em plaquetas em ratos Wistar submetidos ao modelo de compressão de nervo mediano

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ABSTRACT

BACKGROUND AND OBJECTIVES: Platelet-rich fibrin is a new and promising technique to accelerate repair, with possible analgesic effects; however, there is still a gap with regard to peripheral nerve injury and the association with physical exercises. So, this study aimed at evaluating the effects of platelet-rich fibrin associated to physical exercises on nociception and edema in experimental median nerve compression model.
METHODS: Thirty-six rats, all submitted to median nerve compression, were divided in six groups: G1: without additional manipulation; G2: compression and treated with platelet-rich fibrin; G3: compression and treated with free swimming; G4: compression and walking on a treadmill; G5: free swimming + platelet-rich fibrin; G6: walking on a treadmill + platelet-rich fibrin. Injury was induced by tying the median nerve with chrome plated catgut 4.0. Platelet-rich fibrin was obtained by centrifuging 1.5 mL of blood and positioning the fibrin clot directly on the compression region. Exercises were carried out during two weeks, between the 3rd and 14th postoperative days. Nociception and edema were evaluated, respectively, by flinch threshold and plethysmometer, in moments before injury and in the 3rd, 7th and 15th postoperative days.
RESULTS: There have been no differences among groups, only among evaluations, showing increased nociception and edema, which has lasted or improved, respectively, over time.
CONCLUSION: Platelet-rich fibrin alone or associated to physical exercises has not changed nociception and edema.
Keywords: Edema, Exercise therapy, Inflammation, Pain measurement.

RESUMO

JUSTIFICATIVA E OBJETIVOS: Fibrina rica em plaquetas é uma técnica nova e promissora na aceleração do reparo, com possíveis efeitos analgésicos, contudo, ainda há uma lacuna com relação à lesão nervosa periférica, bem como com a associação com exercícios físicos. Assim, o objetivo deste estudo foi avaliar os efeitos da fibrina rica em plaquetas associada a exercício físico sobre a nocicepção e o edema, em modelo experimental de compressão do nervo mediano.
MÉTODOS: Foram utilizados 36 ratos, todos submetidos a compressão do nervo mediano e divididos em seis grupos: G1: sem manipulações adicionais; G2: compressão e tratado com fibrina rica em plaquetas; G3: compressão e tratado com natação livre; G4: compressão e exercício de caminhada em esteira; G5: natação livre + fibrina rica em plaquetas; G6: caminhada em esteira + fibrina rica em plaquetas. O modelo de lesão foi realizado com amarria do nervo mediano, com fio catgut 4.0 cromado. Para obtenção da fibrina rica em plaquetas, 1,5mL de sangue foi centrifugado e o coágulo de fibrina foi posicionado diretamente sobre a região da compressão. Os protocolos de exercício foram realizados durante 2 semanas, entre o 3º e 14º dias de pós-operatório. As avaliações nociceptivas e de edema ocorreram, respectivamente, pelo limiar de retirada de pata e pletismometria, nos momentos prévios à lesão, no 3º, 7º e 15º dias de pós-operatório.
RESULTADOS: Não houve diferenças entre os grupos, apenas entre as avaliações, denotando que houve aumento da nocicepção e do edema, o qual perdurou ou foi decaindo, respectivamente, com o passar do tempo.
CONCLUSÃO: O uso isolado ou associado da fibrina rica em plaquetas com exercícios físicos não produziu alterações na nocicepção e edema.
Descritores: Edema, Inflamação, Mensuração da dor, Terapia por exercício.

INTRODUCTION

Platelets play a critical role in angiogenesis regulation because they are responsible for activation and release of cytokines and growth factors which induce cell proliferation and activation[1].
In addition, platelet concentrates have been used to speed tissue repair due to the high content of platelet-derived growth factor (PDGF), of transforming growth factor beta (TGF-β), of insulin growth factor (IGF) and of vascular endothelium

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growth factor (VEGF)[2].

Platelet-rich fibrin (PRF) is a new autogenous biomaterial which may induce angiogenesis, immune control and increased circulating mesenchymal cells[3]. It has also great potential for routine use to control postoperative (PO) pain and discomfort, being used both to repair bone tissue and soft tissue[4]. In addition, PRF is easy to use and has low cost. There are however controversies about its results[5,6], because there are few evidences of its benefits[4], such as, for example, in peripheral neuropathies.

Most common upper extremity neuropathy is carpal tunnel syndrome (CTS). Such condition is responsible for substantial costs for society in terms of loss of productive capacity and treatment costs[7]. CTS is a condition affecting millions of individuals, causing chronic pain, altered sensitivity and thenar atrophy[8].

First treatment option for CTS is conservative, with the use of braces, local and oral steroids, in addition to physiotherapeutic resources such as physical exercises[7]. There is evidence that exercise-induced analgesia is due to both increased pain threshold and increased blood endogenous opioid levels[9].

Since new therapies are needed to treat peripheral neuropathies, this study aimed at evaluating the effect on nociception and edema of PRF associated to physical exercise in an experimental model of median nerve compression.

METHODS

Experiment was made up of 36 male Wistar rats, mean weight 363.4±59,6g and aged 12±2 weeks, kept in photoperiod of 12h, 24±1 ºC, with free water and food. Animals were randomly divided in six groups, according to treatment:

G1 (n=8) – submitted to nerve compression;
G2 (n=8) – nerve compression + PRF;
G3 (n=8) – nerve compression and free swimming;
G4 (n=8) – nerve compression and treadmill walking exercise;
G5 (n=8) – nerve compression and free swimming + PRF;
G6 (n=8) – nerve compression and treadmill walking exercise + PRF.

Median nerve compression protocol

A tying model with chrome plated Catgut thread 4.0, in four points, with approximate distance of 1 mm in the median nerve, in the proximal region of the right elbow was used to compress the median nerve[10]. For surgical median nerve compression procedure animals were anesthetized with ketamine hydrochloride (85mg/kg) and xylazine hydrochloride (10mg/kg).

The model induces painful symptoms and decreased motor function, which start around the third day and increase nociception around the 7th day.

Protocol to obtain platelet-rich fibrin

To prepare PRF, 1.5 mL of blood was removed from each animal via cardiac puncture[11], which is a safe volume according to Ehrenfest et al.[12]. Immediately after blood removal, sample was placed in sterile eppendorf-type tubes (without anticoagulant) for centrifugation in 3000rpm, with power of approximately 400G during 10 minutes. PRF was removed from the middle layer of the centrifuged sample, between the red part (below) and plasma (above). Fibrin clot was then positioned directly on the compression region of the median nerve for G2, G5 and G6 animals.

Swimming protocol

One treatment modality for animals was low intensity swimming, without overload. For such, animals were placed in a water tank with capacity for 200L, depth of 60cm and water temperature between 30 and 32º C. G3 and G5 animals received swimming stress five days before surgery (to get used to it), interrupting soon after surgery, and restarting in the 3rd until the 14th PO day, in a total of 12 days of therapy. Both during training and during the swimming period, time was 10 minutes/day.

Treadmill walking protocol

Another therapy modality was walking on electric treadmill adapted to rats, with speed of 10m/min, for 10 minutes/day for G4 and G6. Similarly to swimming, there has also been a five-day training period before nerve compression procedure and then again from the 3rd to the 14th PO day.

Evaluation of nociception by flinching threshold

Nociception was evaluated by limb flinching threshold at mechanical stimulation. A Von Frey-type, Insight® brand digital analgesimeter was used for painful sensitivity test. The equipment consists of a transducer arm, with a disposable polypropylene pointer with capacity of 0.1 to 1000g, connected to an amplifier, which measures pressure applied to animal's surface.

Animals were manually contained and filament was applied in two regions: nerve compression and palmar regions. Polypropylene pointer was applied perpendicularly to the area, with gradual pressure increase, and as soon as the animal removed the right foreleg, test was interrupted to record flinching threshold. There has been a three-day period for animals' adaptation and training. Evaluations were performed before injury (AV1) and at beginning of treatment (3rd PO – AV2), 7th (AV3) e 15th PO day (one day after therapy completion – AV4).

Evaluation of edema

Edema formation was evaluated in the distal compression region (distal to wrist) because it may influence clinical animal evolution. For such, a plethysmometer (Insight ®) was used always after flinching threshold evaluation in the same days. At 15th PO day, at the end of evaluations, animals were euthanized.

Statistical analysis

Results were expressed and analyzed by means of descriptive and inferential statistics. Data normality was analyzed with Kolmogorov-Smirnov test and then with mixed Analysis of Variance to decrease the possibility of type II errors by excess of tests; in all cases significance level was 5%.

Project was carried out according to international standards for animal experiment ethics and was approved by the Animal Experiment and Practical Lessons Ethics Committee of the institution under protocol 05612 of 2012.

RESULTS

During nociception evaluation there have been significant differences at nerve compression site ($F(2.3; 69.8)=49.8$, $p<0.001$). Differences were not among groups ($p>0.05$) but rather among evaluations being AV1 higher than remaining evaluations ($p<0.001$). AV4 was significantly higher than AV2 ($p=0.038$) and AV3 ($p=0.002$) (Table 1).

There have also been significant differences in nociceptive evaluation of the palmar region ($F(1.9; 55.9)=14.1$, $p<0.001$). Similar to what has been observed at compression site, differences were not among groups ($p>0.05$) but rather among evaluations, being AV1 higher than remaining evaluations ($p<0.001$) (Table 2).

There have been significant differences in edema evaluation ($F(3; 90)=11.3$, $p<0.001$). Again, differences were not among groups ($p>0.05$) but rather among evaluations, being AV1 lower than AV2 ($p=0.005$) and AV2 higher than AV3 ($p=0.003$) and AV4 ($p>0.001$) (Table 3).

DISCUSSION

PRF has been especially studied and used in dentistry, due to its effects on speeding repair by the release of growth factors[13], and also for being easy to use and prepare[14]. Our study aimed at looking for other ways to use it, such as peripheral nerve injuries; however, regardless of the group, PRF alone or associated to physical exercise has not changed nociception and edema. Lack of positive results was also observed in a study evaluating PRF to cover gingival recesses in the central region of jaws of 10 volunteers[15]. This was also observed with regard to ineffective bone regeneration in chronic periodontitis patients[16]. On the other hand, other authors have reported PRF efficacy on bone regeneration of patients with intraoral bone defects after cystic enucleation[17].

In a study with PRF associated or not to ceramics for calvarium bone repair of rabbits, authors have reported that PRF had positive effect on bone formation when used alone or in combination[2]. Other authors, however, when evaluating the effects of PRF on rotator cuff insertion regeneration of rats submitted to bilateral tenotomy and supraspinous repair, have observed more tensile strength on animals submitted to PRF. Repair characteristic, however, was compatible with fibrovascular tissue, suggesting a possible inhibitory effect of rotator cuff healing[18]. A study evaluating PRF in cavities due to third molar extraction has shown no differences on bone tissue repair, however pain reduction may suggest soft tissue repair[4]. Another study, also with third molar extractions, has shown improved edema without however decreased pain in-

Table 1. Mean and standard deviation of flinching when pressure (gf) was applied to nerve compression site

	G1	G2	G3	G4	G5	G6
AV1	192.5±89.9	267.7±91.7	299.3±66.1	252.6±117.3	287.7±51.3	239.9±62.4
AV2*#	124.8±27.5	117.0±56.6	104.8±29.3	109.0±22.5	119.3±32.1	153.9±33.1
AV3*#	106.7±43.0	118.3±65.3	108.0±47.3	114.7±52.4	143.5±66.4	127.7±52.0
AV4*	116.2±70.8	167.5±119.0	155.5±90.2	193.8±138.0	173.5±93.3	193.4±115.0

*Significant difference as compared to AV1. # Significant difference as compared to AV4.

Table 2. Mean and standard deviation of flinching when pressure (gf) was applied to palmar region

	G1	G2	G3	G4	G5	G6
AV1	188.6±65.8	243.2±55.2	255.6±44.8	278.5±43.1	308.4±43.5	252.0±44.2
AV2*	235.5±70.9	175.4±44.7	203.9±59.9	205.8±30.2	154.8±30.5	202.7±52.1
AV3*	181.4±29.8	175.0±47.5	175.7±18.9	178.7±28.4	164.4±27.1	164.4±40.5
AV4*	176.2±51.0	189.9±106.5	184.9±92.9	162.7±80.8	161.3±47.7	208.0±88.1

*Significant difference as compared to AV1.

Table 3. Mean and standard deviation of limb edema, by plethysmometry (mL)

	G1	G2	G3	G4	G5	G6
AV1	1.56±0.30	1.66±0.26	1.47±0.24	1.34±0.24	1.39±0.46	1.29±0.21
AV2*	1.77±0.23	1.93±0.33	1.81±0.34	1.93±0.61	1.50±0.41	1.73±0.25
AV3#	1.60±0.57	1.39±0.43	1.56±0.13	1.33±0.25	1.32±0.54	1.39±0.35
AV4#	1.36±0.16	1.43±0.36	1.47±0.09	1.39±0.08	1.08±0.48	1.22±0.20

*Significant difference as compared to AV1. # Significant difference as compared to AV2.

tensity[19]. Although mean flinching in G1 being lower than those for other groups, statistical analysis has shown no differences among groups, but only among evaluations.

Our study had several limitations, such as the use of intermediate PRF fraction, which may not have the highest amount of available growth factors[20]; centrifugation time might have been short to be able to potentiate growth factors release, as shown by a recent study which has pointed to advantages of centrifuging for 12 minutes as compared to 10 minutes[21], in addition to the evaluation of just two signs of the inflammation/repair process. It has also to be stressed that PRF associated or not to other modalities is still a new technique with in vitro[22] and in vivo[15-17] studies showing contradictory results, thus requiring further studies with regard to its effects.

CONCLUSION

O uso isolado ou associado da FRP com exercícios físicos não produziu alterações na nocicepção e no edema, no modelo de compressão de nervo mediano.

PRF alone or associated to physical exercises has not changed nociception and edema in a median nerve compression model.

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